**Clostridium herbivorans** sp. nov., a Cellulolytic Anaerobe from the Pig Intestine

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A new cellulolytic anaerobic clostridium was isolated from the intestinal tract of pigs. The single isolate was a gram-positive, motile rod, formed terminal to subterminal swollen sporangia, and required a fermentable carbohydrate for growth. Cellulose, cellobiose, maltose, starch, and glycogen supported growth; but glucose and fructose did not. The major end products from the fermentation of cellobiose were butyrate and formate; minor amounts of hydrogen and ethanol were also formed. Ruminal fluid (15%) or yeast extract (1%) was required for good growth. The optimum temperature for growth was 39 to 42°C, and the optimum pH was 6.8 to 7.2. Cell lysis occurred rapidly once stationary growth was reached. A 16S RNA sequence analysis showed that the strain was related to a group of gram-positive anaerobes that includes *Clostridium oroticum* and the cellulolytic species *Clostridium polysaccharolyticum* and *Clostridium populne*. The DNA base composition of the isolate is 38 mol% G+C. We propose the name *Clostridium herbivorans* for this organism; strain 54408 (= ATCC 49925) is the type strain.

**MATERIALS AND METHODS**

The most probable numbers for the cellulolytic bacterial population in the large intestines of pigs were estimated to be 4 and 6% of the viable bacterial counts when the animals were fed low- and high-fiber diets, respectively (33). Thus, significant quantities of cellulose may be degraded in the large intestines of pigs. The volatile fatty acids produced from this fermentation of cellobiose were butyrate and formate; minor amounts of hydrogen and ethanol were also formed. Ruminal fluid (15%) or yeast extract (1%) was required for good growth. The optimum temperature for growth was 39 to 42°C, and the optimum pH was 6.8 to 7.2. Cell lysis occurred rapidly once stationary growth was reached. A 16S RNA sequence analysis showed that the strain was related to a group of gram-positive anaerobes that includes *Clostridium oroticum* and the cellulolytic species *Clostridium polysaccharolyticum* and *Clostridium populne*. The DNA base composition of the isolate is 38 mol% G+C. We propose the name *Clostridium herbivorans* for this organism; strain 54408 (= ATCC 49925) is the type strain.

**RESULTS AND DISCUSSION**

**Colony and cellular morphology.** Electron microscopy and phase-contrast microscopy showed that cells of strain 54408 1 were motile, straight rods (0.7 to 0.9 by 3.5 to 4.0 μm) that occurred in pairs or as single cells (Fig. 1) (34). The cells were peritrichous, and cells from exponential cultures frequently had 15 to 20 flagella per cell. Spores were rarely observed; however, when spores were present, they were subterminal to terminal and 1 μm wide by 1 to 2 μm long and caused swelling of the cells. When strain 54408 1 was grown in cellulose roll tubes, it produced two zones of clearing (Fig. 2), depend-

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FIG. 1. Transmission electron micrograph of *C. herbivorans*. Cells were fixed with glutaraldehyde, spread on a carbon-coated Formvar grid, and stained with 1% phosphotungstic acid (pH 7). Bar = 1 μm.

...ing on the location of the organism in the agar. If cells were on the surface of the agar, no visible colonies were formed and the zone of clearing was larger than the zone of clearing produced when cells were embedded in the agar. When cells were imbedded in the agar distinct colonies were produced.

**Biochemical reactions.** Growth of strain 5440^T^ was supported by cellulose, cellobiose, maltose, starch, and glycogen. In the presence of cellobiose plus yeast extract the generation time was 2.3 h at 37°C. Strain 5440^T^ did not utilize substrates such as glucose, Casamino Acids, fructose, pectin, sucrose, xylose, and xylan. Other substrates that did not support growth were amygdalin, arabinose, erythritol, galactose, inositol, lactose, lactate, mannoi, mannose, melezitose, melibiose, pyruvate, raffinose, rhhamnose, ribose, sorbitol, and trehalose. Strain 5440^T^ did not digest chopped meat or reduce sulfate or nitrate and was negative for catalase, oxidase, and urease activities. It produced formate (28 mM) and butyrate (12 mM) as major fermentation products when cells were cultured with cellobiose. Ethanol (1 mM) and hydrogen (5 mM) were also produced. Anaerobic conditions were required for growth. The optimum temperature for growth was between 39 and 42°C; however, cultures could be adapted to grow at 45°C. Growth did not occur at 25 or 55°C. The optimum pH for growth was between 6.8 and 7.2. No growth occurred at pH 5.8 or 8.0. We have isolated other strains of this organism, including some strains isolated from Meishan pigs imported from the People's Republic of China. The characteristics of two strains which were obtained from Meishan pigs and which were characterized phenotypically were similar to the characteristics of type strain 5440^T^.

**Cellular fatty acid composition.** The cellular fatty acid composition of strain 5440^T^ was distinct and was not similar to the cellular fatty acid composition of any other bacterium in the gram-positive low-G+C-content phylogenetic cluster (clostridia and clostridium-like organisms) in the MIDI database. The major components identified in strain 5440^T^ were C_{14:0} (43.8% of the total fatty acids), iso-3-OH C_{13:0} (8.9%), C_{16:0} (5.3%), iso C_{14:0} (2.2%), cis-9 C_{18:1} (1.4%), C_{14:0} di-
methyl acetal (22.1%) and C14:0 aldehyde (10.2%). The presence of the two latter components indicated that plasmalogen was present in the cells (11). *C. polysaccharolyticum* was phylogenetically related to strain 54408T (see below) and was analyzed to determine its fatty acid composition since it was not included in the MIDI database. The cellular fatty acid profile of this organism was distinct from that of strain 54408T. The major components identified in *C. polysaccharolyticum* were iso-3-OH C13:0 (24.9% of the total fatty acids), C14:0 (22.1%), iso C16:0 (11.9%), C16:0 (5.9%), C15:0 (5.8%), anteiso C15:0 (2.9%), iso C14:0 (2.2%), C15:0 dimethyl acetal (8.8%), C13:0 anteiso dimethyl acetal (5.1%), and C14:0 aldehyde (3.4%). The presence of the three latter components also indicated that plasmalogen is present in *C. polysaccharolyticum*.

**Phylogeny.** Figure 3 shows the phylogenetic relationship of strain 54408T to other clostridia and related microorganisms, including several mesophilic, cellulytic species (Table 1). This phylogenetic tree, which was prepared by using a maximum-likelihood method (21), is essentially identical topographically to the tree prepared with a distance matrix method (5, 13, 22) previously used by us (data not shown).

Strain 54408T was more closely related to *C. polysaccharolyticum* than to any of the other species whose sequences were deposited in the rRNA database (16). However, the 16S rRNA sequences of strain 54408T and *C. polysaccharolyticum* differ by 3.3%, indicating that these organisms belong to different species (28).

Strain 54408T is a member of a group of clostridia, designated cluster XIVa by Collins et al. (4), which includes "*Acetitomaculum ruminis*" (6), *Clostridium celercrecens*, *C. oroticum*, *Clostridium populeti*, *C. symbiosum*, *E. elgins* (10), and *E. ventriosum*. This group of clostridia is phylogenetically distinct from clostrial rRNA homology group I defined by Johnson and Francis (12), which was called cluster I by Collins et al. (4), was represented in this study by *Clostridium butyricum*, "*Clostridium chartatabidum*", and *Clostridium pasteurianum*, and also includes the cellulolytic species *Clostridium cellulovorans* and "*Clostridium longiporum"*. Clostral rRNA homology group II of Johnson and Francis (12), which was designated cluster XI by Collins et al. (4), is represented in Fig. 3 by *Clostridium lituseburens* and *Clostridium mayombei*. The phylogeny of the other cellulolytic clostridia has been discussed by Collins et al. (4) and was specifically examined by Rainey and Stackebrandt (24). Strain 54408T is phylogenetically distinct from the other mesophilic, cellulytic clostridia.

**Distinguishing characteristics.** Some of the characteristics which distinguish *C. herbivorans* from phylogenetically related species and the mesophilic cellulytic clostridia are listed in Table 1. The closest relative, phylogenetically and phenotypically, of *C. herbivorans* is *C. polysaccharolyticum*. Both species utilize cellulose, celllobiose, maltose, and starch, although they do not utilize glucose. The distinguishing characteristics include G+C content (38 and 42 mol%) and the fermentation of...
arabinose, xylan, and xylose by *C. polysaccharolyticum* but not by *C. herbivorans*. The ecosystems from which these organisms were isolated (pig intestinal tracts and sheep rumina for *C. herbivorans* and *C. polysaccharolyticum*, respectively) are also different. The cell morphologies and peritrichous flagella of these two species are similar, and both organisms are straight rods that occur singly or in pairs. Cells are gram-positive straight rods (0.7 to 0.9 by 3.5 to 4.0 \( \mu m \) wide by 2 \( \mu m \) long and cause the sporangium to swell. Cultures grown with insoluble substrates such as cellulose or plant cell walls more readily produce spores than filaments that are 50 \( \mu m \) long. Long chains and aseptate or more long have been observed in this species.

**Description of Clostridium herbivorans sp. nov.** *Clostridium herbivorans* (her.bi.vo'rans L. fem. n. herba, a green plant; L. v. vario, to devour; M. L. part. adj. herbivarous, devouring plants). Cells are gram-positive straight rods (0.7 to 0.9 by 3.5 to 4.0 \( \mu m \)) that occur in pairs or as single cells. Cells are motile, and peritrichous with 15 to 20 flagella per cell. Cells rarely sporulate; however, when spores are present, they are subterminal to terminal and 1 \( \mu m \) wide by 2 \( \mu m \) long and cause the sporangium to swell. Cultures grown with insoluble substrates such as cellulose or plant cell walls more readily produce spores than those grown with soluble substrates.

**Obligate anaerobe.** Growth requires a fermentable carbohydrate such as cellulose, cellulose, maltose, starch, or glycogen. The following compounds do not support growth: amygdalin, arabinose, Casamino Acids, erythritol, fructose, glucose, inositol, lactate, lactose, mannitol, mannose, melezitose, melibiose, pectin, pyruvate, raffinose, rhamnose, ribose, salicin, sorbitol, sucrose, trehalose, xylose, and xylan. Nitrate and sulfate are not reduced. Catalase, oxidase, and urease negative. Esculin, lecithin, and gelatin are not hydrolyzed. Meat is not digested. Indole is not produced.

The optimum temperature for growth is 39 to 42°C, and the optimum pH for growth is 6.8 to 7.2. Rapid lysis of cells occurs in broth media once the stationary growth phase is reached; this is slightly less true with agar media. Ruminal fluid (15%, vol/vol) and yeast extract (1%, wt/vol) stimulate growth.

The major end products of cellulose fermentation are formate and butyrate; minor amounts of ethanol and hydrogen are also produced. The G+C content of the DNA is 38 mol% (as determined by the buoyant density method).

The type strain, strain 54408, was isolated from intestinal contents of a pig and has been deposited in the American Type Culture Collection as strain ATCC 49925.

**ACKNOWLEDGMENTS**

We thank Greg Strout (Samuel Roberts Noble Electron Microscopy Laboratory) for assistance with the electron microscopy. We thank John L. Johnson and Robert L. Gherna for providing *C. longisparum*, strain 54408, was isolated from intestinal type cultures (30, 31), and have not been seen in *C. herbivorans* cultures.

**C. herbivorans** can be distinguished from all of the remaining mesophilic cellulolytic and phylogenetically related organisms except *Clostridium aldrichii* by its lack of glucose fermentation. *C. herbivorans* and *C. aldrichii* can be clearly differentiated by their fermentation products and fermentation of glycogen, maltose, starch, and xylan. *C. herbivorans* is also distinct from all organisms listed in Table 1 except *C. polysaccharolyticum* in that it does not produce acetate.

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